Amendments to the Specification:

Please replace the paragraph on page 1 under the title "CROSS REFERENCE TO RELATED APPLICATIONS" with the following:

--This application is a divisional application of U.S. Patent Application No. 09/534,811, filed March 24, 2000, which claims priority from provisional application ~~no.~~ No. 60/126,069, filed March 25, 1999~~,~~; the contents of ~~which~~ both applications are hereby incorporated herein by reference.--

Please replace the paragraph beginning at page 6, line 1, with the following:

--The invention also provides inhibitors of GADD45 polypeptide activity in the form of GADD45 peptides comprising sequences which are required for GADD45 activity. These GADD45 peptides, when administered to a cell, interfere with the ability of GADD45 to inhibit a Cdc2/cyclin B1 protein complex from phosphorylating histone H1. In alternative embodiments, the blocking GADD45 peptides comprise amino acids 62-67 of SEQ ID NO:2, and can, for example, have a sequence as set forth by amino acid residues 61 to 87 of SEQ ID NO:2. Alternatively, they can be other subsequences of the GADD45 protein which comprise the DEDDDR (SEQ ID NO:5) acidic motif (amino acid residues 62-67 of GADD45) and a portion of the native sequence of SEQ ID NO:2 flanking that motif on the amino or the carboxyl sides, or both. In various embodiments, the GADD45 peptides can be 10, 20, 30, 40, 50 or 60 amino acid residues in length. These peptides, like the GADD45 binding inhibitors of the invention, can be co-administered with cancer therapeutic agents, particularly those which damage DNA (as, e.g., UV irradiation or chemotherapeutic agents such as cisplatin) to sensitize proliferating cells to the DNA damaging anti-cancer agent. The invention further provides polypeptides which interfere with or inhibit the ability of GADD45 to reduce or eliminate phosphorylation of histone H1. Typically, such polypeptides also comprise the acidic motif DEDDDR (SEQ ID NO:5).--

Please replace the paragraph beginning at page 6, line 26, with the following:

--**Figure 1**. Mapping of the functional domain of GADD45 required for G2/M arrest. (A) Sequence comparison of the human mouse and rat GADD45 family members was made with the multiple alignment module of the DNASTAR software. The GenBank accession numbers of these sequences are as follows: hGADD45 (SEQ ID NO:2), NM_001924; hGADD45β (SEQ ID NO:6), AF078077; hGADD45γ (SEQ ID NO:7), AF078078; mGADD45 (SEQ ID NO:8), B56535; rGADD45 (SEQ ID NO:9), L32591 (h, human; m, mouse; r, rat). (B) The activities of the GADD45 deletion mutants. Different parts of the N-terminus, C-terminus or the central region of GADD45 were deleted. The mutants were introduced into normal human fibroblast cells via microinjection to determine their ability to induce a G2/M arrest. The data are averages from at least three independent experiments. Error bars represent one standard deviation.--

Please replace the paragraph beginning at page 7, line 4, with the following:

--Ability of site mutagenesis mutants to induce a G2/M arrest. (A) Sequence composition of GADD45 between residues 58 and 91. The three regions targeted for mutagenesis are underlined (WT = SEQ ID NO:10). These regions were chosen based on their extensive conservation among the members of the GADD45 family. Site-directed mutagenesis was used to change residues 62-67 (M62-67; SEQ ID NO:11), 74-79 (M74-79; SEQ ID NO:12), or 82-87 (M82-87; SEQ ID NO:13) to alanines. (B) The mutants were expressed in normal primary human fibroblasts via microinjection and the percentage of mutant-expressing cells that arrested at G2/M were determined after 24 h. Results are an average of three separate experiments Error bars represent one standard deviation.--

Please replace the paragraph beginning at page 7, line 30, with the following:

--The present invention demonstrates that GADD45 activates a G2/M checkpoint after damage induced by either UV radiation or alkylating agents, but is not involved in a G2/M checkpoint induced by ionizing radiation. Increased expression of GADD45 in normal human fibroblasts arrests the cells in G2/M. The data presented herein demonstrate that the amino and carboxyl termini of GADD45 are dispensable for the G2/M arrest, but that the central region (residues 50-76) is required. Further, the invention demonstrates that a unique acidic motif, DEDDDR (SEQ ID NO:5), in this region plays a key role in the inhibition of Cdc2/cyclin B1 kinase activity and in the induction of a G2/M arrest. Polypeptides of this acidic motif or comprising this motif can serve as small molecule, dominant negative inhibitors of GADD45 activity.--

Please replace the paragraph beginning at page 9, line 23, with the following:

--Specifically, the invention provides new inhibitors of GADD45 activity in the form of GADD45 peptides comprising "active site" sequences. These GADD45 "active site" peptides interact with sites which are important to GADD45 interactions with other polypeptides, *e.g.*, Cdc2, to interfere with the activity of endogenous GADD45 polypeptide. These "active site" peptides comprise the acidic motif DEDDDR (SEQ ID NO:5) found at residues 62-67 of GADD45 (SEQ ID NO:2), and modified forms of this sequence, and portions of the GADD45 sequence which flank this motif. The "active site" peptides can be subsequences of a GADD45 binding site domain, *e.g.*, in various embodiments, they are 10, 20, 30, 40, 50, and 60 amino acid residues in length. It should be noted that residues 62-67 are involved in GADD45-Cdc2 binding interactions, but are not solely responsible for these interactions. Binding interactions are localized in the domain defined by residues 50 - 76 of SEQ ID NO:2.--

Please replace the paragraph beginning at page 10, line 3, with the following:

--The invention demonstrates that the acidic motif DEDDDR (SEQ ID NO:5) is important in native GADD45 for GADD45-induced G2/M arrest. The acidic nature of this region is conserved among the GADD45 family, including GADD45β and GADD45γ. Acidic regions of these other members of the GADD45 family, however, do not have the ability to block GADD45-induced G2/M arrest.--

Please replace the paragraph beginning at page 10, line 15, with the following:

--Ran, a small nuclear GTPase implicated in both cell cycle progression and nuclear export (Lounsbury, K. M., *et al.*, *J Biol Chem* 271:32834-32841 (1996); Ren, M., *et al. Mol. Cell Biol* 14:4216-4224 (1994)), contains a closely related acidic motif. DEDDDL (SEQ ID NO:14), in its carboxyl-terminal domain. (Richards, S. A., *et al.*, *J Biol Chem* 270:14405-14411 (1995)). Overexpression of Ran also predominantly induces a G2/M arrest, whereas deletion of this acidic motif abolishes such activity. (Ren, M., *et al., Mol. Cell Biol* 14:4216-4224 (1994).) Ran, however, is present in cells in large amounts and its levels do not seem to be regulated by DNA damage or stress. Thus, the studies regarding the presence of an acidic motif in the Ran protein did not offer any suggestion that the acidic motif of that protein could be used to block G2 arrest in cells undergoing DNA damage or stress.--

Please replace the paragraph beginning at page 12, line 31, with the following:

--The term "inhibitor of GADD45 polypeptide activity" refers to a composition capable of specifically interfering with GADD45 polypeptide-related G2/M arrest. More specifically, it refers to compositions comprising a DEDDDR (SEQ ID NO:5) subsequence, encompasses compounds, such as polypeptides, which comprise that subsequence and portions of the sequence of the GADD45 protein (SEQ ID NO:2) which flank that subsequence, particularly the alanine on the amino-terminal side and some or all of the first 20 residues on the carboxy terminal side of DEDDDR (SEQ ID NO:5). GADD45 inhibitors also include, *e.g.*,

antibodies and small molecules which specifically bind to the GADD45 polypeptide and interfere with its binding to or interaction with, a Cdc2/cyclin B1 complex.--

Please replace the paragraph beginning at page 17, line 16, with the following:

--The human GADD45 nucleic acid sequences and other nucleic acids used to practice this invention, whether RNA, cDNA, genomic DNA, or hybrids thereof, may be isolated from a variety of sources, genetically engineered, amplified, and/or expressed recombinantly. Nucleic acid and amino acid sequences of human GADD45 are well known in the art, see, *e.g.*, GenBank Accession Nos. AF078078, AF078077, and M60974. See GenBank Accession No. L24498; and Takekawa (1998) Cell 95:521-530; Papathanasiou (1991) Mol. Cell. Biol. 11:1009-1016. The nucleic acid coding sequence (SEQ ID NO:1 and the amino acid sequence (SEQ ID NO:2) for human GADD45 are:

~~5' ggcagtggct gggaggcagc ggcccaatta gtgtcgtgcg gcccgtggcg aggcgaggtc 61~~
~~cggggagcga gcgagcaagc aaggcgggag gggtggccgg agctgcggcg gctggcacag 121~~
~~gaggaggagc ccgggcgggc gaggggcggc cggagagcgc cagggcctga gctgcccgag 181~~
~~cggcgcctgt gagtgagtgc agaaagcagg cgcccgcgcg ctagccgtgg caggagcagc 241~~
~~ccgcacgccg cgctctctcc ctgggcgacc tgcagtttgc aatatgactt tggaggaatt 301~~
~~ctcggctgga gagcagaaga ccgaaaggat ggataaggtg ggggatgccc tggaggaagt 361~~
~~gctcagcaaa gccctgagtc agcgcacgat cactgtcggg gtgtacgaag cggccaagct 421~~
~~gctcaacgtc gacccccgata acgtggtgtt gtgcctgctg gcggcggacg aggacgacga 481~~
~~cagagatgtg gctctgcaga tccacttcac cctgatccag gcgttttgct gcgagaacga 541~~
~~catcaacatc ctgcgcgtca gcaacccggg ccggctggcg gagctcctgc tcttggagac 601~~
~~cgacgctggc cccgcggcga gcgagggcgc cgagcagccc ccggacctgc actgcgtgct 661~~
~~ggtgacgaat ccacatttat ctcaatggaa ggatcctgcc ttaagtcaac ttatttgttt 721~~
~~ttgccgggaa agtcgctaca tggatcaatg ggttccagtg attaatctcc ctgaacggtg 781~~
~~atggcatctg aatgaaaata actgaaccaa attgcactga agtttttgaa atacctttgt 841~~
~~agttactcaa gcagttactc cctacactga tgcaaggatt acagaaactg atgccaaggg 901~~
~~gctgagtgag ttcaactaca tgttctgggg gccccggagat agatgacttt gcagatggaa 961~~
~~agaggtgaaa atgaagaagg aagctgtgtt gaaacagaaa aataagtcaa aaggaacaaa 1021~~
~~aattacaaag aaccatgcag gaaggaaaac tatgtattaa tttagaatgg ttgagttaca 1081~~
~~ttaaaataaa ccaaatatgt taaagtttaa gtgtgcagcc atagtttggg tatttttggt 1141~~

~~ttatatgccc tcaagtaaaa gaaaagccga aagggttaat catatttgaa aaccatattt 1201~~

~~tattgtattt tgatgagata ttaaattctc aaagttttat tataaattct actaagttat 1261~~

~~tttatgacat gaaaagttat ttatgctata aatttttga aacacaatac ctacaataaa 1321~~

~~ctggtatgaa taattgcatc att - 3' (SEQ ID NO:1)~~

~~5' MTLEEFSAGE QKTERMDKVG DALEEVLSKA LSQRTITVGV YEAAKLLNVD~~

~~PDNVVLCLLA ADEDDDRDVA LQIHFTLIQA FCCENDINIL RVSNPGRLAE~~

~~LLLLETDAGP AASEGAEQPP DLHCVLVTNP HSSQWKDPAL SQLICFCRES~~

~~RYMDQWVPVI NLPER 3' (SEQ ID NO:2).~~

5'-ggcagtggct gggaggcagc ggcccaatta gtgtcgtgcg gcccgtggcg aggcgaggtc 61

cggggagcga gcgagcaagc aaggcgggag gggtggccgg agctgcggcg gctggcacag 121

gaggaggagc ccgggcgggc gaggggcggc cggagagcgc cagggcctga gctgccggag 181

cggcgcctgt gagtgagtgc agaaagcagg cgcccgcgcg ctagccgtgg caggagcagc 241

ccgcacgccg cgctctctcc ctgggcgacc tgcagtttgc aatatgactt tggaggaatt 301

ctcggctgga gagcagaaga ccgaaaggat ggataaggtg ggggatgccc tggaggaagt 361

gctcagcaaa gccctgagtc agcgcacgat cactgtcggg gtgtacgaag cggccaagct 421

gctcaacgtc gaccccgata acgtggtgtt gtgcctgctg gcggcggacg aggacgacga 481

cagagatgtg gctctgcaga tccacttcac cctgatccag gcgttttgct gcgagaacga 541

catcaacatc ctgcgcgtca gcaacccggg ccggctggcg gagctcctgc tcttggagac 601

cgacgctggc cccgcggcga gcgagggcgc cgagcagccc ccggacctgc actgcgtgct 661

ggtgacgaat ccacattcat ctcaatggaa ggatcctgcc ttaagtcaac ttatttgttt 721

ttgccgggaa agtcgctaca tggatcaatg ggttccagtg attaatctcc ctgaacggtg 781

atggcatctg aatgaaaata actgaaccaa attgcactga agtttttgaa ataccttttgt 841

agttactcaa gcagttactc cctacactga tgcaaggatt acagaaactg atgccaaggg 901

gctgagtgag ttcaactaca tgttctgggg gcccggagat agatgacttt gcagatggaa 961

agaggtgaaa atgaagaagg aagctgtgtt gaaacagaaa aataagtcaa aaggaacaaa 1021

aattacaaag aaccatgcag gaaggaaaac tatgtattaa tttagaatgg ttgagttaca 1081

ttaaaataaa ccaaatatgt taaagtttaa gtgtgcagcc atagtttggg tatttttggt 1141

ttatatgccc tcaagtaaaa gaaaagccga aagggttaat catatttgaa aaccatattt 1201

tattgtattt tgatgagata ttaaattctc aaagttttat tataaattct actaagttat 1261

tttatgacat gaaaagttat ttatgctata aatttttga aacacaatac ctacaataaa 1321

ctggtatgaa taattgcatc att - 3' (SEQ ID NO:1)

MTLEEFSAGE QKTERMDKVG DALEEVLSKA LSQRTITVGV YEAAKLLNVD PDNVVLCLLA ADEDDDRDVA LQIHFTLIQA FCCENDINIL RVSNPGRLAE LLLLETDAGP AASEGAEQPP DLHCVLVTNP HSSQWKDPAL SQLICFCRES RYMDQWVPVI NLPER (SEQ ID NO:2).--

Please replace the paragraph beginning at page 18, line 27, with the following:

--The compounds of the invention include peptides from or derived from the GADD45 amino acid sequence, that contain the DEDDDR (SEQ ID NO:5) acidic motif, and which are capable of interfering with the interaction of GADD45 protein and Cdc2, with the ability of GADD45 to dissociate a Cdc2/cyclin B1 complex, or both. The full length amino acid sequence of human wild type GADD45 is shown in SEQ ID NO:2, above. The full-length protein, of course, interacts with Cdc2 and has normal GADD45 activity, and is not within the scope of the invention herein. The polypeptides of the invention are portions of the GADD45 protein or conservative variations thereof which have activity to modulate GADD45 activity as measured by the assays taught herein, but do not themselves have GADD45-like ability to cause dissociation of a Cdc2/cyclin B1 complex, inhibit a Cdc2/cyclin B1 complex from phosphorylating histone H1, or both. In preferred embodiments, the polypeptides reduce or eliminate the ability of GADD45 to reduce the phosphorylation of histone H1 by a Cdc2/cyclin B1 complex.--

Please replace the paragraph beginning at page 19, line 7, with the following:

--In preferred forms, the compounds of the invention include the acidic motifs DEDDDR (SEQ ID NO:5) or DEDDDRD (SEQ ID NO:15) found at positions 62-68 and 62-69, respectively, of the wild-type GADD45 sequence. Peptide compositions of the invention include not only these specific peptide sequences, but also fragments of the GADD45 protein that contain these motifs and which retain the ability to interfere with GADD45-related dissociation of Cdc2/cyclin B1 complexes. The motifs may also have non-essential moieties attached to the

peptides. The term "non-essential moieties", as used herein, refers to those chemical moieties that do not prevent the peptide from inhibiting GADD45-related reduction of the phosphorylation of histone H1. In this context, "non-essential moieties" refers to additional residues or substituents that do not significantly alter the biological properties of the peptides, e.g., their ability to compete with GADD45 in interacting with Cdc2 in Cdc2/cyclin B1 complexes. For example, the term "non-essential moieties" includes amino acid sequence extensions at either the amino-terminal or carboxy-terminal end of the acidic motifs which do not prevent these peptides from inhibiting the interaction of GADD45 and Cdc2.--

Please replace the paragraph beginning at page 19, line 22, with the following:

--Where specific peptide subsequences of a larger protein subsequence are demonstrated to have the requisite properties of the protein, it is apparent to those of skill that the addition of amino acids to the critical peptide is non-essential material. They are preferably added in the natural order (native order) in which they are found in GADD45 as depicted in SEQ ID NO:2. The nonessential material can be added to either end or terminus of the given peptide. These peptides have an amino and carboxy terminus to which additional amino acids can be added. Examples of such amino acid sequence extensions include the portions of the naturally occurring amino acid sequence of GADD45 protein depicted in SEQ ID NO:2 on either side of the acidic motifs. Preferably, such amino acid extensions are no longer than about 25 amino acids in length at either that C-terminal or amino terminal end of the motifs, and more preferably are about 20 amino acids in length or shorter. Because amino acid residues 61 to 87 of SEQ ID NO:2 have been shown to be particularly important in inducing G2/M arrest, more preferred forms of the peptides of the invention contain this sequence (which has some 20 residues extending beyond the acidic motif on the carboxy end). Fewer residues beyond the carboxy end of the acidic motif may, however, be used. For example, the carboxy end can be extended 5, 10, or 15 residues beyond the end of the acidic motif. The ability of any such peptides to inhibit

GADD45-related dissociation of Cdc2/cyclin B1 complexes can be determined by the methods described herein. Exemplary peptides or polypeptides of the invention include the following:

EAAKLLNVDPDNVVLCLLAADEDDDRDVALQIHFTLIQAFCCENDI (SEQ ID NO:16;

LLNVDPDNVVLCLLAADEDDDRDVALQIHFTLIQAFCC (SEQ ID NO:17);

DNVVLCLLAADEDDDRDVALQIHFTL (SEQ ID NO:18);

CLLAADEDDDRDVALQIHFTL (SEQ ID NO:19);

DNVVLCLLAADEDDDRDVALQ (SEQ ID NO:20);

EAAKLLNVDPDNVVLCLLAADEDDDR (SEQ ID NO:21);

DEDDDRDVALQIHFTLIQAFCCENDI (SEQ ID NO:22);

ADEDDDRDVALQIHFTLIQAFCCENDI (SEQ ID NO:23);

ADEDDDRDVALQIHFTL (SEQ ID NO:24);

ADEDDDRDVALQ (SEQ ID NO:25);

DEDDDR (SEQ ID NO:5);

ADEDDDR (SEQ ID NO:26);

DNVVLCLLAADEDDDR (SEQ ID NO:27);

CLLAADEDDDR (SEQ ID NO:28);

CLLAADEDDDRD (SEQ ID NO:29);

CLLAADEDDDRDVAL (SEQ ID NO:30);

DNVVLCLLAADEDDDRDVALQIHFTLIQAFCCENDI (SEQ ID NO:31); and

LNVDPDNVVLCLLAADEDDDRDVALQIHFTLIQAFCCENDI (SEQ ID NO:32).--

Please replace the paragraph beginning at page 22, line 9, with the following:

--The methods of the invention use anti-GADD45 polyclonal and monoclonal antibodies directed against GADD45 polypeptides and peptides, particularly those directed against portions of SEQ ID NO:2 which are involved GADD45 activity, such as amino acid residues 62-68 (the DEDDDR (SEQ ID NO:5) acidic motif) and against portion of GADD45

involved in protein:protein binding with Cdc2, such as those defined by the region between amino acid residues 50-76 of SEQ ID NO:2.--

Please replace the paragraph beginning at page 23, line 28, with the following:

--The invention provides *in vitro* and *in vivo* methods of assaying for a modulator of GADD45 polypeptide activity by identifying molecules that specifically bind the GADD45 polypeptide, thereby affecting its activity. While the invention is not limited by what means the GADD45 polypeptide activity is inhibited, specific embodiments include assaying for GADD45 polypeptide binding to Cdc2, its ability to dissociate a Cdc2/Cyclin B1 complex and inhibit the complex's kinase activity, and others, as described herein. One embodiment includes targeting the active site and protein:protein binding domain of the GADD45 polypeptide, which is described for the first time herein, as discussed above. Additionally, the DEDDDR (SEQ ID NO:5) acidic motif can be targeted. The methods of the invention also include screening for antibodies directed to GADD45 or small molecule binders of GADD45 polypeptides. To assay for specific binding of a putative modulatory molecule, the GADD45 polypeptide can be in solution or can be attached to a fixed substrate. In one embodiment, GADD45 polypeptide is fixed to a solid substrate for high throughput screenings and column chromatography.--

Please replace the paragraph beginning at page 34, line 12, with the following:

--The invention provides modulators (*e.g.*, inhibitors) of GADD45 polypeptide activity and their therapeutic administration. Modulators that can be used therapeutically also include antibodies and small molecules which bind to GADD45 to inhibit its ability to bind Cdc2, or its ability to dissociate a Cdc2/Cyclin B1 complex, or its ability to inhibit Cdc2/cyclin B1 complex phosphorylation of histone H1. In another embodiment, the modulator is a peptide inhibitor of GADD45 activity comprising a GADD45 active site peptide or a protein:protein binding domain peptide (*e.g.*, based on amino acid residues 62-67 or residues 50-76 of SEQ ID

NO:2, respectively) which can specifically associate with a GADD45-binding site on another protein (*e.g.*, Cdc2). These compounds include those found by the methods of the invention and peptides and polypeptides, such as DEDDDR (SEQ ID NO:5) or peptides containing a DEDDDR (SEQ ID NO:5) motif, which inhibit GADD45 polypeptide activity. In one embodiment, the peptides, polypeptides and other compositions of the invention are administered with a pharmaceutically acceptable carrier(s) (excipient) to form the pharmacological composition.--

Please replace the paragraph beginning at page 56, line 13, with the following:

--The central region (residues 50-76) contains many acidic residues. To examine if the acidic residues play an important role in GADD45-mediated G2/M arrest, three additional mutants, M62-67, M74-79 and M82-87, were made in which the residues within these regions were changed to alanines by site-directed mutagenesis. The M82-87 mutant is similar to wild-type GADD45 with regard to its ability to induce a G2/M arrest in normal human fibroblasts, while M74-79 partially lost activity and M62-67 was completely deficient. These data indicate that the DEDDDR (SEQ ID NO:5) motif within residues 62-67 is critical for GADD45-mediated G2/M arrest.--

Please replace the paragraph beginning at page 59, line 9, with the following:

--These findings suggest that the G2/M checkpoint may utilize a redundant system. It has been reported that GADD45 interacts with the nuclear proteins $p21^{wafl}$ and PCNA. (Kearsey, J. M., *et al.*, *Oncogene* 11:1675-1683 (1995); Smith, M. L., *et al.*, *Science* 266:1376-1380 (1994).) GADD45 can disrupt the ability of $p21^{wafl}$ to bind to PCNA, and, conversely, $p21^{wafl}$ blocks the ability of GADD45 to bind to PCNA. (Chen, I. T., *et al.*, *Oncogene* 11:1931-1937 (1995).) Although the central region (residues 50-76) of GADD45 is required for interaction with both Cdc2 and $p21^{wafl}$, the mutant M62-67 that lost its inhibitory

effect on the Cdc2/cyclin B1 kinase can still bind to Cdc2 and $p21^{wafl}$. Moreover, $p21^{wafl}$ is not required for GADD45-induced G2/M arrest. (Wang, X. W., *et al.*, *Proc. Natl. Acad. Sci U.S.A.* 96:3706-3711 (1999).) Therefore, the binding of GADD45 to Cdc2 and $p21^{wafl}$ is insufficient to define the mechanism of a GADD45-mediated G2/M checkpoint. The additional activity of the DEDDDR (SEQ ID NO:5) motif is needed to inactivate the Cdc2/cyclin B1 kinase and to induce a G2/M arrest. In addition, the GADD45-mediated G2/M checkpoint is independent of 14-3-3σ because overexpression of GADD45 is still able to induce a G2/M arrest in 14-3-3σ-deficient HCT116 cells (gift of Dr. Bert Vogelstein). These data indicate that three p53 downstream genes may utilize different mechanisms to activate the G2/M checkpoints.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 13, at the end of the application.